Hairless is required for the development of adult sensory organ precursor cells in Drosophila

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Summary

Reduction of the wild-type activity of the gene Hairless (H) results in two major phenotypic effects on the mechanosensory bristles of adult Drosophila. Bristles are either 'lost' (i.e. the shaft and socket fail to appear) or they exhibit a 'double socket' phenotype, in which the shaft is apparently transformed into a second socket. Analysis of the phenotypes conferred by a series of H mutant genotypes demonstrates (1) that different sensilla exhibit different patterns of response to decreasing levels of H+ function, and (2) that the 'bristle loss' phenotype results from greater loss of H+ function than the 'double socket' phenotype. The systematic study of H allelic combinations enabled us to identify genotypes that reliably produce specific mutant defects in particular positions on the bodies of adult flies. This permitted us to investigate the cellular development of sensilla in these same positions in larvae and pupae and thereby establish the developmental basis for the mutant phenotypes. We have found that H is required for at least two steps of adult sensillum development. In positions where 'double socket' microchaetes appear on the notum of H mutant flies, sensillum precursor cells are present in the developing pupa and divide normally, but their progeny adopt an aberrant spatial arrangement and fail to differentiate correctly. In regions of the notum exhibiting 'bristle loss' in adult H mutants, we were unable at the appropriate stages of development to detect sensillum-specific cell types, the precursor cell divisions that generate them, or the primary precursor cells themselves. Thus, the H 'bristle loss' phenotype appears to reflect a very early defect in sensillum development, namely the failure to specify and/or execute the sensory organ precursor cell fate. This finding indicates that H is one of a small number of identified genes for which the loss-of-function phenotype is the failure of sensillum precursor cell development.

Key words: Drosophila, Hairless, peripheral nervous system, sensillum development, cell fate.

Introduction

Organized in a precise pattern over the integument of adult Drosophila is an elaborate array of epidermally derived sensory organs, or sensilla. Though these sensilla are morphologically diverse, and provide various types of sensory information to the fly, all are composed of a small number of distinct cell types. For example, a typical mechanosensory sensillum ('bristle') includes a single bipolar neuron and three different accessory cells; the latter form cellular sheaths around the dendrite of the neuron and produce the stimulus-receiving cuticular structures of the bristle organ. The bristle shaft is the product of the trichogen cell, while the socket surrounding the base of the shaft is made by the tormogen cell.

For at least some types of adult sensilla, the cells constituting an individual sensillum are generated during pupal development via a fixed lineage from a single precursor cell (Hartenstein and Posakony, 1989). Thus, a primary step in sensillum development, which takes place in the imaginal discs and histoblast nests during the late larval and early pupal stages, is the selection of sensillum precursors from an undifferentiated cell population, the remainder of which will become epidermal cells. Following the divisions of the precursor cells, the presumptive sensillum cells undergo a characteristic series of morphogenetic and differentiative changes to give rise to a mature sensory organ. In order to understand the genetic pathways and cellular mechanisms that underlie these developmental events, we are investigating the specific role of genes required for normal sensillum patterning and/or cytodifferentiation.

Loss-of-function mutations of Hairless (H) confer two distinct mutant phenotypes on the bristle sensilla of adult Drosophila (see Lindsley and Grell, 1968; Lindsley and Zimm, 1985). Bristles are either 'lost' (i.e. the shaft and socket fail to appear) or they exhibit a 'double socket' phenotype, in which the shaft is apparently transformed into a second socket (Lees and Waddington, 1942). Earlier studies have provided some information concerning the developmental basis of these effects. Lees and Waddington (1942) showed that
the 'double socket' effect is associated with a failure of the trichogen cell to assume its normal subepidermal position. Nash (1965) presented evidence that the defect underlying the 'bristle loss' phenotype occurs earlier in bristle development than that which results in a 'double socket'. Besides these effects on sensory bristles, H also causes shortening of wing veins, particularly the fourth and fifth longitudinal veins, and enlargement of the eye.

H mutations have also been shown to exhibit phenotypic interactions with mutant alleles of the neurogenic genes Notch (N), Delta (Dl), Enhancer of split [E(spl)], neuralised (neu), and mastermind (mam). Strong H loss-of-function alleles suppress the adult wing phenotypes of N and Dl, and enhance the bristle loss and wing vein shortening of Abruptix (Ax) alleles of N, as well as the bristle and eye defects of the split (spl) allele of N (see Lindsay and Grell, 1968; Lindsay and Zimm, 1985). Other interactions of H with the neurogenic genes have been studied by Campos-Ortega and colleagues (Dietrich and Campos-Ortega, 1984; Vässin et al. 1985; Knust et al. 1987; de la Concha et al. 1988). In the compound eyes of mosaic adults, H rescues the cell lethality of a strong loss-of-function allele of DI and reduces the severity of defects caused by homozygosity for neuF65 (Dietrich and Campos-Ortega, 1984). H and the E(spl)D gain-of-function mutation interact synergistically to reduce the number of sensilla at the anterior margin of the wing (Knust et al. 1987). Vässin et al. (1985) and de la Concha et al. (1988) investigated the effects of H+ dosage on the phenotypes caused by mutant alleles or altered dosages of several neurogenic genes. In the embryo, H suppresses the neural hyperplasia resulting from homozgyosity for loss-of-function alleles of N, Dl, neu, and mam, but not E(spl), suggesting that among the neurogenic genes E(spl) may be the major target of H function (Vässin et al. 1985; de la Concha et al. 1988).

These phenotypic effects and genetic interactions of H mutations suggest that the wild-type gene plays an important role in adult peripheral nervous system development in Drosophila. Consequently, we have undertaken a genetic and molecular investigation of H function. Here we report the results of a detailed analysis of adult sensory organ development in H mutants, with particular emphasis on the mechanosensory bristles of the notum.

Materials and methods

Fly stocks

Flies were cultured on standard yeast–cornmeal–molasses–agar medium at 25°C. Mutant alleles of Hairless (H; 3–69.5) used in this study are listed in Table 1. The dominant phenotypic effects of strong H alleles (see Lindsay and Grell, 1968; Lindsay and Zimm, 1985) are due to haploinsufficiency, as shown by the segmental aneuploidy analysis of Lindsay et al. (1972). In addition, with one exception, previously described H alleles are recessive lethals. Hf and Hw were discovered by Sturtevant and are described in Lindley and Grell (1968). The alleles C19, C20, C23, C25, C28, and RP1 were recovered by R. Groger and J. W. P. in a γ-ray mutagenesis as dominant suppressors of the third chromosome mutation Bearded (Brd; M. Leviten and J. W. P., unpublished data) and/or on the basis of their H dominant phenotype; the chromosomes carrying these alleles were marked with the eye color mutation pink-peach (pP). Xf/ and Hf/ were X-ray induced (kindly provided by M. Brand and J. A. Campos-Ortega). We have found that H/KX is associated with a T(2;3) translocation, with breakpoints at 41 and 92F1-2. Bf (kindly provided by U. Tepass and J. A. Campos-Ortega) was isolated in a P element mutagenesis. Cytological analysis shows that the Hf/ chromosome carries a deficiency with breakpoints at 92B3-11 and 92F8-13. Our conclusion that this deficiency deletes the H locus is based on the following evidence. Segmental aneuploidy has grossly localized H between 92C and 94A4 (Lindsay et al. 1972). As noted by Ashburner (1982), ebony (e) deficiencies that do not uncover H define a distal limit of 93B for the location of H. Vässin et al. (1985) described three H+ duplications with proximal breakpoints in 92E, further defining the locus proximally. The most precise cytological location available for H is apparently 92F1-2, as suggested by the third chromosome breakpoint of the translocation associated with H/KX; this location is well within the boundaries of the region deleted cytologically by Df(3R)B79. It should be noted that de la Concha et al. (1988) have described an embryonic phenotype associated with a H deficiency; however, no cytological information concerning this deficiency was provided.

The transposon insertion P[lac,ry+]A37 (Ghysen and O'Kane, 1989; kindly provided by Y. Hiromi and C. O'Kane) was used as a marker for sensory organ precursor cells and presumptive sensillum cells in the wing imaginal disc (A. G. B. and J. W. P., unpublished data). For this purpose, recombinant chromosomes of the following genotypes were constructed: A37 Hf e1 and A37 Hf P/1. Apparently because of a nuclear localization signal encoded by the P transposase-lacZ fusion gene in the A37 transposon, β-galactosidase protein and activity become localized to the nucleus (Bell et al. 1989).

Chromosomes and marker mutations not described herein are described in Lindley and Grell (1968) and in Lindley and Zimm (1985, 1990).

Table 1. H mutations used in this study

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Origin</th>
<th>Cytology</th>
</tr>
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<tbody>
<tr>
<td>Strong (Hf)</td>
<td>Spontaneous</td>
<td>Normal</td>
</tr>
<tr>
<td>Hf</td>
<td>Spontaneous</td>
<td>Normal</td>
</tr>
<tr>
<td>Hf/T</td>
<td>γ-ray</td>
<td>Normal</td>
</tr>
<tr>
<td>Hf/E</td>
<td>γ-ray</td>
<td>Normal</td>
</tr>
<tr>
<td>Hf/28</td>
<td>γ-ray</td>
<td>Normal</td>
</tr>
<tr>
<td>Hf/KM</td>
<td>X-ray</td>
<td>Normal</td>
</tr>
<tr>
<td>Hf/XI</td>
<td>X-ray</td>
<td>T(2; 3)41; 92F1-2</td>
</tr>
<tr>
<td>Weak (Hf)</td>
<td>γ-ray</td>
<td>Normal</td>
</tr>
<tr>
<td>Hf/P</td>
<td>γ-ray</td>
<td>Normal</td>
</tr>
<tr>
<td>Deficiency</td>
<td>Pelement</td>
<td>Df(3R)92B3-11; 92F8-13</td>
</tr>
</tbody>
</table>

H alleles were classified as strong (Hf) or weak (Hf) based on their own phenotype and on their behavior in crosses inter se (see Materials and methods and Results).
Identification of \( H \) mutant larvae and pupae

All \( H \) mutant chromosomes used in this study were maintained in \textit{trans} to the balancer chromosome TM6B, \( Hu e Tb ca \) (Craymer, 1984; Lindsley and Zimm, 1990). TM6B is marked with the dominant mutation Tubby (\( Tb \)), which causes the animal to appear short and fat, especially in the larval and pupal stages. This enabled us to distinguish unambiguously \( H \) homozygote and transheterozygote larvae and pupae from those carrying the balancer. Survival of \( H \) mutant animals to pupation could therefore be readily scored, and \( H \) larvae and pupae of defined genotype could easily be identified for use in microscopy and staining experiments.

Electron microscopy

Procedures for transmission electron microscopy of pupal imaginal disc tissues were as described (Hartenstein and Posakony, 1989). Adult flies were prepared for scanning electron microscopy as follows. Whole flies were dehydrated overnight in isopropanol acetate, air dried and mounted on aluminum stubs with double-stick tape. Specimens were then coated with gold or gold/palladium and examined and photographed with a Hitachi S-405A or a Cambridge S360 scanning electron microscope.

Antibody labeling and application of bromodeoxyuridine (BrdU)

Monoclonal antibodies 22C10 and 21A6 (Zipursky et al. 1984; kindly provided by S. Benzer), and a monoclonal antibody against BrdU (Beckton-Dickinson) were used in this study. Procedures for both antibody labeling and application of BrdU were as described (Hartenstein and Posakony, 1989).

We have observed that in preparations doubly labeled with anti-BrdU and MAb 22C10, the labeling of sensillum cell bodies by 22C10 was sometimes weak; labeling of neurons (particularly axons), however, remained strong (see Fig. 6A–D and Fig. 8).

Histochemical staining for \( \beta \)-galactosidase activity

Histochemical demonstration of \( \beta \)-galactosidase expression from the transposon insertion \( P[\text{lac,ry}^+]A37 \) was carried out as described by Romani et al. (1989).

Results

Phenotypic classification of \( \text{H} \) alleles

As described in the Introduction, \( H \) loss-of-function mutations are associated with two dominant phenotypic effects on adult sensory bristles (see Lindsley and Grell, 1968; Lindsley and Zimm, 1985). The first is the apparent replacement of the bristle shaft with a second socket, resulting in a characteristic ‘double socket’ cuticular structure (see Figs 1 and 5). The second is the complete absence of the cuticular apparatus of the bristle (‘bristle loss’). In addition, with one exception (Albornoz, 1984), previously described \( H \) alleles are recessive lethals (Lindsley and Grell, 1968; Lindsley and Zimm, 1985).

We observed that our initial collection of ten mutant alleles of \( H \) could be classified as strong (\( H^S \)) or weak (\( H^W \)), based on their heterozygous and homozygous phenotypes and their behavior in crosses \textit{inter se} (Table 1; see Materials and methods). By these criteria, an allele in one group was found to be indistinguishable from other alleles in that group. Among the strong group, the cytologically normal alleles \( H^2 \) and \( H^3 \) (Lindsley and Grell, 1968) were used for the experiments in this study. These alleles appear to be null mutations, based on our observation that pharate adults of the genotypes \( H^W/\text{Df}(3R)B79 \) and \( H^W/\text{Df}(3R)B79 \) are identical in phenotype to pharate adults homozygous for \( H^2 \) or \( H^3 \) (see following section). \( \text{Df}(3R)B79 \) is a cytologically visible deficiency for the \( H \) locus (see Materials and methods). Thus, by standard functional definitions (Muller, 1932), \( H^2 \) and \( H^3 \) are apparent amorphs, as are the other members of the strong group.

As demonstrated by the results presented in the following section, the two alleles in the weak group (Table 1) are hypomorphic for \( H \) function; they closely resemble the \textit{Hairless}-recessive (\( H^7 \)) allele described by Albornoz (1984).

A \( H \) phenotypic series

As illustrated in Fig. 1, the different possible genotypic combinations of \( H^S \), \( H^W \) and \( H^7 \) alleles create a phenotypic series of increasingly severe mutant defects, in the following order: \( H^S/+ \ll H^W/+ \ll H^W/H^W \ll H^W/H^S \). By using heteroallelic combinations for both \( H^W/H^W \) and \( H^W/H^W \) genotypes, we could make it more likely that the mutant defects that we observe are attributable to \( H \) and are not caused by unrelated recessive mutations on the mutant chromosomes.

\( H^W \) mutations are almost completely recessive: \( H^W/+ \) flies are most frequently wild type in phenotype, but occasionally a ‘double socket’ appears on the head in the position of a postvertical macrochaeta (Fig. 1A,A’) or on the abdominal tergites. \( H^W/+ \) flies, by contrast, exhibit the fully penetrant \( H \) haploinsufficient phenotype (Lindsley and Grell, 1968; Lindsley and Zimm, 1985; Lindsley et al. 1972). Macrochaetes of the head, notum and abdominal tergites are most sensitive to the effects of \( H^W \) alleles, and many of these bristles exhibit either the ‘double socket’ or the ‘loss’ phenotype in \( H^W/+ \) flies (Fig. 1B,B’).}

\( H^W/H^W \) flies display a much stronger and more extensive mutant phenotype than either \( H^W \) or \( H^W \) heterozygotes (Fig. 1C,C’). In addition to the loss of many macrochaetes on the head and notum, approximately 50\% of the notum microchaetes are missing (\( n=8 \) nota). The remaining 50\% exhibit a spectrum of ‘double socket’ phenotypes, ranging from a partial to a complete transformation of shaft into socket (see Fig. 5).

The phenotypically most severe \( H \) allelic combination which is still adult viable is \( H^W/H^W \). Approximately 50\% of flies of this genotype die as pharate adults. The remainder survive to eclosion, but are short-lived as adults. They exhibit extensive loss of both macrochaetes and microchaetes on the head, notum and abdominal tergites (Fig. 1D,D’). Only 20\% of the notum microchaetes remain (\( n=8 \) nota), all with a completely transformed ‘double socket’ phenotype. Nevertheless, ‘double sockets’ frequently remain in the positions of the posterior scutellar macrochaetes (Fig. 1D).
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We have observed that the $H^P/H^P$ genotype is lethal during larval and pupal stages, contrary to a previous report that these animals die as embryos (de la Concha et al. 1988). We did find that homozygotes of various $H^C$ alleles survive to pupation at relatively low frequencies (from <1% to 10% of expected). However, for $H^P/H^P$ heteroallelic combinations the frequency of animals surviving to pupal stages reaches 60–80% of expected, presumably because chromosome-specific recessive lethal effects have been eliminated. These animals were
dissected from their pupal cases for examination and found to be completely devoid of macrochaetes and microchaetes on the head and notum (Fig. 1E,E'), with occasional 'double sockets' remaining on the abdominal tergites. The only bristles that appear to be significantly resistant to loss of \( H^+ \) function are those on the legs, where many 'double sockets' and some normal bristles remain.

On the basis of these results, we interpret the \( H^w \) alleles as hypomorphs, representing partial loss of \( H^+ \) function. In addition, we believe that our observations of \( H^w/H^+ \) animals define the zygotic null phenotype for \( H \).

The 'bristle loss' phenotype results from greater loss of \( H^+ \) function than the 'double socket' phenotype

We analyzed quantitatively the phenotypic effects of \( H \) mutations on the macrochaetes of the head and notum (Table 2 and Fig. 2). Two important conclusions can be drawn from these data. First, different macrochaetes exhibit very different patterns of sensitivity to increasing loss of \( H^+ \) function, so that increasing numbers of bristles are affected as \( H^+ \) function is reduced. Second, the frequency with which a given bristle is completely removed rather than exhibiting a 'double socket' phenotype becomes greater with increasing loss of \( H^+ \) function. This indicates that the normal developmental process that is disrupted to give a 'double socket' requires more \( H^+ \) activity (as a fraction of the wild-type level) than the process that is disrupted to give the 'bristle loss' phenotype.

### Table 2. Quantitation of the phenotypic effects of increasing loss of \( H^+ \) function on head and notum macrochaetes

<table>
<thead>
<tr>
<th>Macrochaetes</th>
<th>( H^w/H^+ )</th>
<th>( H^+/H^+ )</th>
<th>( H^+/H^w )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%dbl</td>
<td>%abs</td>
<td>%dbl</td>
</tr>
<tr>
<td>Anterior orbital (a or)</td>
<td>20</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Medial orbital (m or)</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Posterior orbital (p or)</td>
<td>68</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>Ocellar (oc)</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Inner vertical (i v)</td>
<td>20</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Outer vertical (o v)</td>
<td>63</td>
<td>0</td>
<td>82</td>
</tr>
<tr>
<td>Postvertical (pv)</td>
<td>95</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Upper humeral (u h)</td>
<td>80</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lower humeral (l h)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Presutural (p s)</td>
<td>55</td>
<td>8</td>
<td>50</td>
</tr>
<tr>
<td>Anterior notopleural (a np)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Posterior notopleural (p np)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Anterior supraalar (a sa)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Posterior supraalar (p sa)</td>
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<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Anterior postalar (a pa)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Posterior postalar (p pa)</td>
<td>5</td>
<td>50</td>
<td>13</td>
</tr>
<tr>
<td>Anterior dorsocentral (a dc)</td>
<td>53</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>Posterior dorsocentral (p dc)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anterior scutellar (a sc)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Posterior scutellar (p sc)</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

The phenotypes of the macrochaetes on 20 flies of each indicated genotype (a total of 40 of each macrochaete per genotype) were scored as normal, 'double socket' (dbl), or shaft and socket absent (abs). \( H^w/H^2; H^+/H^w \). A schematic representation of these data is given in Fig. 2.

**Other phenotypic effects of \( H \) mutations**

Although among adult sensory organs the macrochaetes are generally the most sensitive to loss of \( H^+ \) function, other types of sensilla are affected as well, especially in the more severe mutant genotypes \( H^w/H^+ \) and \( H^+/H^w \). Fig. 3 illustrates several examples, including the interommatidial bristles of the eye, the dorsal pedicellar sensilla of the haltere, the campaniform sensilla along the third longitudinal wing vein, the triple row of the anterior wing margin (which bears chemosensory as well as mechanosensory bristles), and the bristles of the female terminalia. All are strongly affected by loss of \( H^+ \) function. Some of these sensilla, such as the triple row bristles and the female anal and genital bristles, exhibit both 'double socket' and loss phenotypes; the others appear to exhibit primarily the loss phenotype. [It should be noted that our finding of interommatidial bristle loss in \( H^w/H^+ \) mutants contrasts with the report of Dietrich and Campos-Ortega (1984) that \( H^w \) somatic clones do not affect the eye.]

We have also studied additional \( H \) mutant phenotypes not involving sensory bristles. As with the bristle defects, the shortening of longitudinal wing veins near the wing margin becomes more severe with increasing loss of \( H^+ \) function (Fig. 4). Enlargement and/or mild roughening of the eye is also observed; these defects become considerably more penetrant in \( H^w/H^+ \) and \( H^+/H^w \) genotypes. Counts of ommatidia per eye in \( H^w/H^+ \) flies indicate that the eye enlargement results from the presence of supernumerary facets (data not shown). We also observed a phenotype not previously described for \( H \); namely, shortening or loss of the non-innervated hairs along the posterior wing margin.
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The developmental basis for H bristle mutant phenotypes

The study of H allelic combinations enabled us to identify genotypes that reliably produce specific mutant defects in particular positions on the cuticle of adult flies. We could then investigate the cellular development of sensilla in these same positions in larvae and pupae, and thereby establish the developmental basis for the mutant phenotypes.

The H 'double socket' phenotype

In a bristle organ expressing the full H 'double socket' phenotype, the trichogen cell fails to produce a shaft and instead produces a second socket-like structure similar to that produced by the tormogen cell. However, this phenotype is not an all-or-none effect of reduction of $H^+$ function. Scanning electron microscopy reveals that in H hypomorphic mutants (e.g. $H^w/H^w$) the cuticular structures produced by the trichogen cells of different bristles range from a defective shaft to a structure having both shaft and socket characteristics to an entirely socket-like structure (Fig. 5). The 'true' socket (that produced by the tormogen cell) is also abnormal, as it fails to completely encircle the structure made by the trichogen cell, even if that structure has retained a significant amount of shaft character. Nevertheless, as in the wild-type bristle, the thickest part of the socket structure made by the tormogen cell always lies posterior to the structure produced by the trichogen cell; in addition, the latter structure retains the anterior–posterior polarity of the wild-type bristle shaft.

The normal differentiation of notum microchaetes (Hartenstein and Posakony, 1989) starts with the subepidermal segregation of the sensory neuron, which sends out an apical dendrite and a basal axon. The cell bodies of the thecogen and trichogen also shift subepidermally, but the tormogen cell body remains in the epidermal plane. Subsequently, the three accessory cells form concentric sheaths around the dendrite; the inner, middle and outer sheaths are produced by the thecogen, trichogen and tormogen, respectively. Finally, the accessory cells produce the stimulus-receiving apparatus of the sensillum, which besides the shaft and socket includes a dendritic cap made by the thecogen. All four microchaete cells can be labeled with the MAb 22C10. The neuron, thecogen and trichogen become 22C10-positive early, before sensillum morphogenesis starts [18–20 h after puparium formation (APF)]; the tormogen is labeled only much later in development (36–40 h APF). A second antibody, MAb 21A6, strongly labels the dendritic cap, and more weakly labels the tormogen cell late in microchaete differentiation.

Antibody staining and transmission electron microscopy of H pupal nota revealed that 'double socket' microchaetes are composed of the normal number of cells and that the initial steps of morphogenesis of these...
Sensillum development requires Hairless

Fig. 3. Scanning electron microscope and light microscope photographs showing the phenotypic effects of H mutant genotypes on various sensory and non-sensory structures of the adult fly. Scanning electron micrographs show dorsal pedicellar sensilla of the haltere of (A) wild type and (B) $H^2/H^3$; interommatidial bristles of the compound eye of (C) wild type and (D) $H^2/H^3$; bristles of the female genitalia and anal plate of (E) wild type and (F) $H^2/H^3$. Light microscope photographs show the distal campaniform sensilla (arrows) of the dorsal third longitudinal wing vein of (G) wild type and (H) $H^2/H^RPI$; the stout mechanosensory bristles of the anterior wing margin (medial triple row) of (I) wild type and (J) $H^2/H^RPI$ (not shown: the chemosensory bristles and slender mechanosensory bristles of the dorsal and ventral triple rows are either lost or exhibit a 'double socket' phenotype); non-innervated epidermal hairs of the posterior wing margin of (K) wild type and (L) $H^2/H^RPI$. Scale bars: A,B: 5 μm; C,D: 10 μm; E,F: 30 μm; G–L: 20 μm.
cells appear normal. Thus, immediately following the final precursor cell divisions, the presumptive neuron and thecogen cell, and a third cell which is almost certainly the trichogen, become 22C10-positive (data not shown), and the cell body of the presumptive neuron shifts subepidermally. The thecogen cell body also shifts subepidermally, and the inner sheath around the dendrite is formed (Fig. 7E,F); the tormogen cell body remains in the epidermal plane. At this point, 'double-socket' microchaete development deviates from normal development. Most strikingly, the trichogen cell body remains at the epidermal surface, instead of shifting to a subepidermal position. Thus, both the trichogen and tormogen cells of a 'double socket' microchaete lie in the plane of the epidermal layer (Fig. 6A,B; Fig. 7A,B) [as previously reported by Lees and Waddington (1942)], and the tormogen cell body is now interposed between the tormogen cell and the thecogen cell’s sheath around the dendrite. One important consequence of this altered cellular configuration is that the outer cytoplasmic sheath formed by the tormogen cell is incomplete, as indicated by the fact that the socket structure that this cell produces no longer fully encircles the structure made by the trichogen (see Figs 5 and 7G). Two additional specific markers were used to assay the expression of tormogen cell characteristics by the 'double socket' trichogen cell. We found that, whereas in normal microchaetes only the tormogen cell is labeled by MAb 21A6 (Hartenstein and Posakony, 1989), both the trichogen and tormogen cells of 'double socket' microchaetes are 21A6-positive (Fig. 6G,H). However, in another respect, the 'double socket' trichogen cell remains unlike a tormogen cell: it exhibits a significantly lower level of β-galactosidase expression from the A37 transposon insertion than the tormogen cell (Fig. 6E,F).

Although as in the wild type the neuron and the thecogen cell bodies shift subepidermally, the dendrite and the dendritic cap (the latter produced by the thecogen cell) are in contact with the epidermal surface. In at least some 'double socket' microchaetes, the dendritic cap is defective apically, so that the dendrite, like a true cilium, protrudes beyond the outer surface of the epidermis (Fig. 7C,D). In both normal and 'double socket' bristles, the dendritic cap is 21A6-positive and is positioned within the cuticular structure produced by the trichogen cell; in the normal bristle, the dendritic cap sits beneath the shaft, while in the 'double socket' bristle the dendritic cap is surrounded by the socket-like product of the trichogen cell. The position of the dendritic cap relative to the trichogen cell body of a 'double socket' bristle is very similar to that which it occupies relative to the tormogen cell body of a normal bristle (Fig. 6G,H).

In order to study the division pattern of the precursor cells that give rise to 'double socket' microchaetes, we employed the technique of pulse-labeling replicating DNA of dividing sensillum precursor cells with the base analogue bromodeoxyuridine (BrdU). In the dorso-central region of the notum, few epidermal cells divide during the period of microchaete precursor cell division (14–18 h APF). Consequently, the division pattern of the microchaete precursor cells is readily analyzed by BrdU pulse-labeling, using MAb 22C10 as a marker for the different sensillum cells (Hartenstein and Posakony, 1989). In the wild type, an individual primary precursor cell divides to give rise to two secondary
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precursor cells. One secondary precursor divides at about 16 h APF to yield the trichogen and tormogen cells; the other divides 1–2 h later to give the neuron and thecogen cells.

Our results with H mutant pupae indicate that the pattern of precursor cell division giving rise to the cells of an individual 'double socket' microchaete is normal (Fig. 6A–D). First, the developmental timing of 'double socket' precursor divisions is the same as in the wild type, since BrdU injections in the interval from 14 to 18 h APF label microchaete cell nuclei in the mutant organs. Second, the observation that trichogen/tormogen nuclei can be labeled independently from neuron/thecogen nuclei in 'double socket' microchaetes indicates that, as in the wild type, the divisions of the two secondary precursor cells are not simultaneous. Finally, as shown in Fig. 6C and D, our finding of labeled, presumably late-replicating, nucleoli in trichogen and tormogen cells of certain 'double socket' microchaetes with heavily labeled neuron and thecogen nuclei suggests that, as in the wild type, the division of the trichogen/tormogen secondary precursor precedes that of the neuron/thecogen precursor.

The H 'bristle loss' phenotype

The failure of the cuticular apparatus of a bristle to appear on the surface of the adult fly, as in the H 'bristle loss' phenotype, indicates only that the trichogen and tormogen cells have failed to differentiate properly. Consequently, we investigated the cellular basis for this mutant phenotype in detail. Using MAb 22C10 as a specific marker for sensillum cells in whole mounts of pupal nota (Hartenstein and Posakony, 1989), we first determined that the H 'bristle loss' phenotype is characterized by the absence of all sensillum cells. In H pupal nota at 22–27 h APF [i.e. 4–9 h after the completion of the microchaete precursor divisions, toward the end of the morphogenetic phase and early in the differentiative phase of microchaete cellular development (Hartenstein and Posakony, 1989)], no 22C10-positive cells appear at positions from which bristles would be missing in adult H flies of the same genotype (Fig. 8). Examination of 22C10-stained H pupal whole mounts of later stages (32–40 h APF, when wild-type microchaete organs are almost fully differentiated) confirmed that not only are the shaft- and socket-forming trichogen and tormogen cells missing (as suggested by the cuticular phenotype), but the neuron and thecogen cell as well (data not shown).

These observations indicated that the defect responsible for the H 'bristle loss' phenotype occurs early in microchaete development, and prompted us to ask whether we could detect DNA replication in dividing microchaete precursor cells in regions of the notum exhibiting absence of (22C10-positive) sensillum cells. The injection of BrdU into H mutant pupae during the time when microchaete precursors normally replicate DNA and divide (i.e. 14–18 h APF) demonstrated that no detectable replication occurs at positions from which microchaetes are lost (Fig. 8). These BrdU pulse-labeling experiments also revealed that in the Hm/Hp genotype there is an overproliferation of epidermal cells restricted to the midline area of the pupal notum, an aberrant pattern that we have never observed at any point during wild-type pupation (Fig. 8B).

Since in developing H mutant pupae neither sensillum-specific cell types nor sensillum precursor cell divisions could be detected in regions of the notum that exhibit 'bristle loss' in adult flies, we next sought to determine whether sensillum primary precursor cells are present at these positions. For this purpose, we employed the β-galactosidase-expressing P element transposon insertion A37; we have obtained strong evidence that, as suggested by Ghysen and O’Kane (1989), A37 is a marker for the primary precursor cells of adult sensilla (A. G. B. and J. W. P., in preparation).
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Fig. 6. Light microscope analysis of the H 'double socket' phenotype. (A–D) Light microscope photographs of a preparation of a H^{PP}/H^{CC} pupal notum, injected with BrdU at 16 h APF, and then fixed and doubly labeled with MAb 22C10 and anti-BrdU antibody at 32 h APF. (A) Normally differentiated microchaete. The trichogen (shaft) cell lies beneath the tormogen (socket) cell; only the tormogen (to) cell is visible in this plane of focus. (B) Microchaete exhibiting the 'double socket' phenotype. The trichogen (tr) and tormogen (to) cells lie side-by-side in the epidermal plane; both are visible in the same plane of focus. The nuclei of both the trichogen and tormogen cells of a 'double socket' microchaete are much larger than those of the surrounding epidermal cells, indicating that, as in the wild type, they have undergone rounds of endoreplication. (C,D) Single 'double socket' microchaete visualized at two different planes of focus. In C, the neuron (ne) and thecogen cell (th) exhibit strong nuclear labeling by anti-BrdU. In D, a more shallow plane of focus shows the side-by-side arrangement of the trichogen and tormogen cells in the epidermal plane; arrows indicate nucleoli labeled with anti-BrdU. General nuclear DNA replication occurs before nucleolar replication. We interpret the labeling patterns shown in these panels to mean that the secondary precursor cell that produces the trichogen and tormogen cells of a 'double socket' microchaete divides before the secondary precursor that produces the neuron and thecogen, just as in wild-type microchaetes. (E,F) Light microscope photographs of nuclei of wild-type (E) and H 'double socket' (F) microchaete cells, showing β-galactosidase expression from the A37 transposon insertion. Pupal nota were dissected, fixed and stained for β-galactosidase activity at 24 h APF. In a wild-type background, all four microchaete cells are positive for A37 β-galactosidase expression immediately following the completion of the precursor cell divisions; as differentiation progresses, expression becomes restricted to the tormogen cell (E; A. G. B. and J. W. P., unpublished data; see also Ghysen and O'Kane, 1989). In a H^{PP}/H^{PP} background (F), the nuclei of both the trichogen (tr) and tormogen (to) cells of a 'double socket' microchaete (lying side-by-side in the epidermal plane) are labeled. The 'double socket' trichogen cell exhibits a significantly lower level of β-galactosidase expression than the tormogen cell. (G,H) Light microscope photographs of normal (G) and 'double socket' (H) vertical macrochaetes on the head of a H^{PP}/H^{PP} pupa, fixed at 40 h APF and labeled with MAb 21A6, which in wild-type sensilla strongly labels the dendritic cap (the product of the thecogen cell) and more weakly labels the tormogen cell. Arrows indicate the dendritic cap, labeled with 21A6. In G, the dendritic cap is positioned within the socket made by the tormogen cell and beneath the shaft made by the trichogen cell, and only the tormogen cell is labeled by the antibody. In H, the dendritic cap is positioned within the socket-like structure made by the trichogen cell, and both the trichogen and tormogen are 21A6-positive.

Discussion

H is required for at least two steps of adult sensillum development

The bristle sensilla of the adult fly exhibit two major abnormalities in response to reduction of H^+ function, the 'double socket' and 'bristle loss' phenotypes. We have investigated the developmental basis for both of these mutant phenotypes in the case of the macro- and microchaetes of the notum. We have shown that, as...
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Fig. 7. Ultrastructural analysis of the H 'double socket' phenotype. Shown are oblique sections of pupal notum preparations at 32 h APF. (A,C,E) $H^{RP1}/H^{RP1}$; (B,D,F) wild type. (A) Trichogen and tormogen cells of a 'double socket' microchaete. They are located in the same plane and show a ruffling of their apical membrane which is absent in normal microchaete cells of a corresponding stage. (B) Wild-type trichogen (tr) and tormogen (to) cells. The cell body of the trichogen is located at a more basal level; at this level it is completely surrounded by the mesaxon-like sheath process of the tormogen. (C) Dendrite (d) of a 'double socket' microchaete which apically breaks through the sheath provided by the thecogen cell (th). In a normal microchaete (D), the tip of the dendrite, surrounded by the dendritic cap (dc) secreted by the thecogen cell, is embedded in the trichogen cell (tr). E and F represent more basal sections, demonstrating that in both H 'double socket' microchaetes (E), as well as in normal microchaetes (F), the dendrite (d) is surrounded by a cylindrical sheath provided by the thecogen cell (th). (G) Schematic representations of the cellular architecture of wild-type and H 'double socket' microchaetes (see Results for description). Both longitudinal and transverse views are shown. Symbols: d, dendrite; dc, dendritic cap; ne, neuron; sh, shaft; so, socket; th, thecogen; to, tormogen; tr, trichogen. Scale bars: 3μm.
Fig. 8. Absence of microchaete precursor cell divisions and sensillum-specific cell types in regions of the wing imaginal disc exhibiting ‘bristle loss’ in H mutants. In the dorsocentral region of the notum, epidermal cells do not divide during the period of microchaete precursor cell division (14–18 h APF). This allows us to examine by BrdU labeling the division pattern of the microchaete precursor cells, in combination with MAb 22C10, a specific marker for sensillum cell types (Hartenstein and Posakony, 1989). Wild-type (A) and H²/H²RPi (B) pupae were injected with BrdU at 16 h APF. At 27 h APF (well after the onset of microchaete differentiation), pupae were dissected, fixed and doubly labeled with MAb 22C10 and anti-BrdU antibody. In positions exhibiting microchaete ‘loss’ in H²/H²RPi adult flies, neither microchaete precursor cell division nor sensillum-specific cell types are detectable (B). Arrows in panel B indicate positions of 22C10-positive cells of the few microchaetes that remain on the notum of adults of this genotype (see Fig. 1D). Note the over-proliferation of epidermal cells along the notal midline in B, an aberrant phenotype we have never observed in wild-type nota.

originally proposed by Nash (1965), the ‘double socket’ phenotype represents a defect in sensillum cell differentiation, while the ‘bristle loss’ phenotype reflects a much earlier defect, at the time of sensillum precursor cell specification.

*Abnormal differentiation is associated with an altered arrangement of sensillum cells in the ‘double socket’ phenotype*

We have found that, in positions where ‘double socket’ microchaetes appear on the notum of H mutant flies, sensillum precursor cells are present in the developing pupa and divide normally, but their progeny fail to differentiate correctly. The most striking abnormality is the apparent transformation of the shaft-producing trichogen cell into a socket-producing ‘tormogen-like’ cell (Lees and Waddington, 1942); this is reminiscent of several lineage mutants of *C. elegans* in which sister cells that normally express different fates now adopt the same fate (Sternberg, 1990). The trichogen cell takes on at least three characteristics of a tormogen: its cell body fails to shift subepidermally, it produces a cuticular structure which resembles a socket and it is 21A6-positive. Nevertheless, the transformation of cell fate is incomplete, in that the ‘double socket’ trichogen cell retains at least two notable characteristics of wild-type trichogen cells: It is 22C10-positive early in its differentiation, and it expresses a significantly lower level of β-galactosidase from the A37 transposon insertion than the tormogen cell.

In a ‘double socket’ microchaete, the trichogen and tormogen cells fail to complete normally one or more of the following: sheath formation, subepidermal segregation and production of the appropriate part of the stimulus-receiving apparatus of the bristle. These steps could represent independent developmental events, each requiring H* function. Alternatively, they could be causally interrelated. Thus, for example, the failure of the ‘double socket’ trichogen cell to shift subepidermally could result in its failure to produce a shaft-like apical process and the inability of the now displaced tormogen cell to form a complete apical sheath around the trichogen. Recently we have obtained evidence that cell–cell interaction plays an important role in adult sensillum cell differentiation in *Drosophila* (Hartenstein and Posakony, 1990). Thus, it is also possible that the inappropriate position and morphology of the ‘double socket’ trichogen cell disrupts the cell–cell contacts required for the proper differentiation of the bristle organ. Only further analysis will reveal which
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**Fig. 9.** Absence of sensillum primary precursor cells and their progeny in regions of the wing imaginal disc exhibiting 'bristle loss' in *H* mutants. (A) Fate map of the third instar wing imaginal disc [adapted from Bryant (1975)]. Symbols for notum macrochaetes are as in Table 2. Other structures are indicated as follows: WM, wing margin; DRS and VRS, campaniform sensilla of the dorsal and ventral radius [Sc4d, Sc25, Sc12 (DRS) and Sc4v, Sc3, Sc5 (VRS) of Bryant (1978)]. (B,C) Light microscope photographs of whole mounts of A37 (B) and A37 *H^{RPE} e^{1}/A37 H^{2} e^{11} (C) late third instar wing imaginal discs, stained for β-galactosidase activity expressed from the A37 transposon insertion. Putative precursor cells for certain wing and notum sensilla are indicated: DRS and VRS, as above; PSC, posterior scutellar macrochaete.

**β-galactosidase-positive cells** present in the wild-type disc (B) are absent from the *H* disc (C) in positions exhibiting the 'bristle loss' phenotype in *H^{+}/H* adults. Specifically, stained cells (presumably macrochaete precursors) are absent in most parts of the notum; only the posterior scutellar macrochaete remains detectable, consistent with its frequent appearance as a 'double socket' in adults of this genotype (see Fig. 1D). Similarly, most putative precursor cells for campaniform sensilla of the dorsal and ventral radius are absent, suggesting that this is the basis for the strong reduction in the numbers of these sensilla in wings of *H^{+}/H* adults (data not shown).

(D-G) Whole-mount preparations of A37 (D,F) and A37 *H^{RPE} e^{1}/A37 H^{2} e^{11} (E,G) pupal nota at 14h (D,E) and 24h (F,G) APF, stained for β-galactosidase. Posterior scutellar macrochaetes are marked with arrows (bottom of each panel); anterior is at the top. At 14h APF (D,E), the macrochaete precursor cells have completed their divisions and their progeny are well differentiated, but the microchaete primary precursor cells are just beginning to divide (Hartenstein and Posakony, 1989). We have found the pattern of smaller β-galactosidase-positive nuclei in the dorso-central region of the notum shown in D to be highly reproducible; we believe these represent microchaete primary precursor cells, based on their positions, their time of appearance, and their division pattern as followed with this and other 'enhancer detector' markers (A. G. B. and J. W. P., in preparation; see Hartenstein and Posakony, 1989). Few microchaete primary precursor cells are observed in the *H* notum (E) compared to the wild-type notum (D); this is evidently the basis for the extensive microchaete 'loss' phenotype of the *H^{+}/H* genotype (see Fig. 1D). Nevertheless, the posterior scutellar macrochaetes are detectable as expected (see Fig. 1D). At 24h APF (F,G), the microchaete precursor divisions have been completed, and the microchaete cells are undergoing differentiation (Hartenstein and Posakony, 1989). The A37 marker displays a dynamic staining pattern in individual sensillum cells as they differentiate, and eventually its expression becomes restricted to the tormogen cell (Ghysen and O'Kane, 1989; A. G. B. and J. W. P., in preparation). In the *H* notum (G), β-galactosidase-positive cells are absent from positions that exhibit complete microchaete 'loss' in *H^{+}/H* adults; those that do appear are in positions consistent with the small number of 'double socket' microchaetes that remain on the notum of these flies (see Fig. 1D). This result confirms our finding that no MAb 22C10-positive (sensillum) cells appear in pupal nota at positions from which bristles are missing in *H* mutant adults (see Fig. 8).

sensillum cells must be *H*+ for normal sensillum differentiation and whether *H* acts cell autonomously or non-autonomously.

It is interesting to note that the mutation *shaven* (sv) causes the formation of bristles with abnormal shafts, which sometimes resemble the *H* 'double socket' (A.
This finding indicates that the Achaete-scute complex (García-Bellido and Santamaria, 1978). Subsequently, it appears that differences in developmental timing alone are insufficient to account for the patterns of sensitivity to mutations that we have observed. Adult bristles exhibit characteristic patterns of differential sensitivity to mutations in several other genes, including the Ax alleles of N (our unpublished observations), the Achaete allele of extramacrochaetae (H. M. Ellis and J. W. P., in preparation), and various loss-of-function mutations of the achaete–scute complex (Stern, 1968; García-Bellido, 1979; Ruiz-Gómez and Modolell, 1987). Although the sensitivity patterns for these mutations are overlapping, they are nonetheless distinct. Perhaps this phenomenon reflects regional differences in the activities of genes involved in sensory organ patterning.

Although the apparent H null genotype (what we have referred to here as H+/H+) results in the loss of almost all adult sensilla, we observed nevertheless that many leg bristles develop normally in such animals. It is possible that the apparent insensitivity of some leg bristles to loss of H+ activity is due to a low residual level of H+ function in the strong alleles that we have characterized as nulls based on standard genetic tests (Muller, 1932). Since Df(3R)B79 homozygotes do not survive to the pupal stage, we have not had the opportunity to determine whether leg bristles appear in whole animals carrying a physical deletion of the H gene; due to the fact that many genes are deleted by the B79 deficiency, we have also not attempted to generate somatic cell clones homozygous for a H deletion. The interpretation that we currently favor is that another gene (or genes) exists whose function is partially redundant with that of H.

The 'bristle loss' phenotype results from greater loss of H+ function than the 'double socket' phenotype

We have found that the 'double socket' phenotype represents both a higher level of H+ activity (as a fraction of wild-type) and a later developmental defect than the 'bristle loss' phenotype. Thus, the 'double socket' phenotype results when the level of H+ function is sufficient to permit the determination and division of the sensillum precursor cell but less than that required for the later differentiation of the sensillum cells themselves. When the level of H+ activity further drops below a certain threshold, the developmental program of the bristle is halted earlier, at the time of appearance of the primary precursor cell, and the 'bristle loss' phenotype results. It is possible that the differential sensitivities documented in Table 2 and Fig. 2 indicate that different bristles have different characteristic H+ activity thresholds for the two mutant defects. This analysis can also be extended to account for the 'double sockets' in which the cuticular product of the trichogen cell retains some shaft-like character; these may result from a level of H+ function intermediate between that necessary for the development of a normal bristle and that which results in the full 'double socket' phenotype.

Though our experiments indicate that two different steps of adult sensillum development require H+ function, they do not address the question of when the H gene product is expressed or is active. It is reasonable to suggest that the 'bristle loss' and 'double socket'
phenotypes reflect two distinct times of \( H \) action; however, it is also possible that both effects derive from reduction of \( H^+ \) function in the sensillum precursor cell. If \( H \) does function at two different times, then an additional point must be considered. We have found that the absence of primary precursor cells is the null phenotype for the action of \( H \) early in sensillum development. It is important to emphasize that we do not know whether the 'double socket' phenotype represents the null condition for the differentiative function of \( H \); complete loss of \( H^+ \) activity at this later time might result in a very different syndrome of differentiative defects. This problem could be approached experimentally through the use of a strong conditional allele of \( H \), which would allow us to analyze the different times of \( H^+ \) requirement separately, as we and others have done for \( N \) with the temperature-sensitive allele \( N^{90} \) (Shellenberger and Mohler, 1978; Cagan and Ready, 1989; Hartenstein and Posakony, 1990).

Other developmental effects of \( H \)

The other mutant effects of \( H \), including enlarged eyes, shortened wing veins and defects in the non-innervated hairs on the posterior margin of the wing, indicate that \( H \) is required for developmental events other than sensillum patterning and differentiation. The wing vein defect is particularly noteworthy, in that several other mutations that cause the gain or loss of adult sensory organs [including the \( Ax \) alleles of \( N \) (see Lindsley and Grell, 1968; Lindsley and Zimm, 1985; Palka et al., 1990) and both gain-of-function (\( Achaetous; H. M. Ellis and J. W. P. \), in preparation) and loss-of-function (Garcia-Alonso and Garcia-Bellido, 1988) alleles of \( extramacrochaetae \)] also cause the gain or loss, respectively, of wing vein tissue. Moreover, both sensillum precursors and wing vein precursors exhibit temporal patterns of cell proliferation distinct from that of typical epidermal cells in the wing imaginal disc (Schubiger and Palka, 1987; Hartenstein and Posakony, 1989). It appears that sensillum patterning and wing vein patterning rely at least in part on common pathways of genetic control.

Finally, a potentially important observation from the BrdU pulse-labeling experiments is that in \( H^+/H^- \) animals there is an overproliferation of epidermal cells along the midline of the pupal notum. It is possible that disturbances in normal patterns of cell proliferation underlie one or more of the developmental defects caused by \( H \).

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